AD-A099 271 ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/5
EFFECTS OF FRANCISELLA TULARENSIS, SALMONELLA TYPHIMURIUM AND S--ETC(U)
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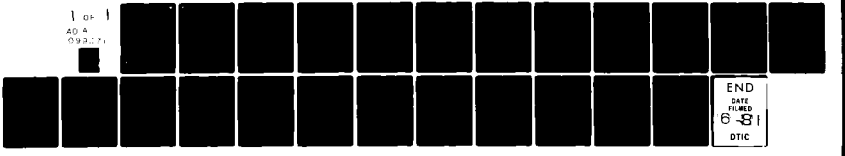

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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
	AD-A099 271	

4. TITLE (and Subtitle)
Effects of Francisella tularensis, Salmonella typhimurium and Streptococcus pneumoniae Infections on Oxidative, Glycolytic and Lysosomal Enzyme Activity in Red and White Skeletal Muscle

5. TYPE OF REPORT & PERIOD COVERED

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Goran Friman, Nils-Gunnar Ilback and William R. Beisel

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
US Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, MD 21701

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
S101AQ-197
871-BB-132
DA 0J6419

11. CONTROLLING OFFICE NAME AND ADDRESS
US Army Medical Research and Development Command, Office of The Surgeon General
Department of the Army, Washington, D.C. 20314

12. REPORT DATE
30 March 1981

13. NUMBER OF PAGES
17

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
Information regarding reprints are not available at this time
Manuscript was submitted to Infection and Immunity for consideration of publication

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Muscle Enzymes	Salmonella
Infections	Francisella
Bacteria	Tularemia
Pneumococcus	
Streptococcus	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Since opinions differ as to whether the oxidative and glycolytic capabilities of skeletal muscle are altered in acute infection, the activities of two oxidative and one glycolytic enzyme were determined in homogenates of skeletal muscle of rats. Groups were inoculated with Francisella tularensis 72 h prior to study or with Salmonella typhimurium or Streptococcus pneumoniae 48 h before enzyme analysis, for comparison with noninfected controls. The activities of six lysosomal enzymes (acid hydrolases) and protein and DNA concentrations

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were also measured. All determinations were made separately on red (slow twitch) and white (fast twitch) muscle tissue, because these muscle fiber types function during different types of exercise.

In the tularemia- and Salmonella-infected rats, the oxidative enzymes of muscle were decreased to 56 to 83% and the glycolytic enzyme to 30 to 75% of control activities. Reductions during tularemia were statistically correlated with whole-cell protein degradation, while that of the glycolytic enzyme was paralleled by activation of lysosomal enzymes. In the pneumococcal infection, only reduced glycolytic activity was significant. Muscle DNA concentrations were unchanged in any infection.

Thus, in representative bacterial infections, glycolytic enzyme situated in the cytosol showed an earlier and relatively more pronounced loss of activity than did the intramitochondrially located oxidative enzymes. Since red and white muscle tissues responded similarly, the mechanisms or pathways for mediating these responses in infection appeared to be independent of those properties that constitute the difference between muscle fiber types. In contrast, in several other physiologic or pathologic states that involve a catabolic response in muscle, predominant catabolism (or damage) usually occurs in one or the other fiber types.

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Short title: SKELETAL MUSCLE TISSUE ENZYMES IN BACTERIAL INFECTION

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The USAMRIID facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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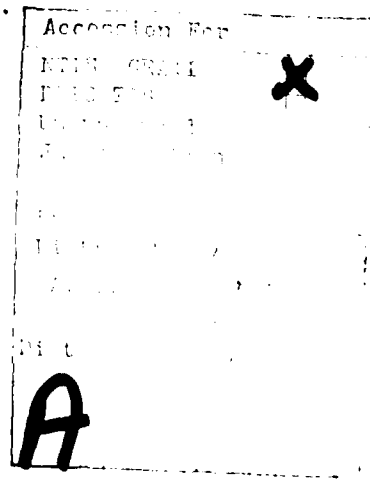

ABSTRACT

Since opinions differ as to whether the oxidative and glycolytic capabilities of skeletal muscle are altered in acute infection, the activities of two oxidative and one glycolytic enzyme were determined in homogenates of skeletal muscle of rats. Groups were inoculated with Francisella tularensis 72 h prior to study or with Salmonella typhimurium or Streptococcus pneumoniae 48 h before enzyme analysis, for comparison with noninfected controls. The activities of six lysosomal enzymes (acid hydrolases) and protein and DNA concentrations were also measured. All determinations were made separately on red (slow twitch) and white (fast twitch) muscle tissue, because these muscle fiber types function during different types of exercise.

In the tularemia- and Salmonella-infected rats, the oxidative enzymes of muscle were decreased to 56 to 83% and the glycolytic enzyme to 30 to 75% of control activities. Reductions during tularemia were statistically correlated with whole-cell protein degradation, while that of the glycolytic enzyme was paralleled by activation of lysosomal enzymes. In the pneumococcal infection, only reduced glycolytic activity was significant. Muscle DNA concentrations were unchanged in any infection.

Thus, in representative bacterial infections, glycolytic enzyme situated in the cytosol showed an earlier and relatively more pronounced loss of activity than did the intramitochondrially located oxidative enzymes. Since red and white muscle tissues responded similarly, the mechanisms or pathways for mediating these responses in infection appeared to be independent of those properties that constitute the difference between muscle fiber types. In contrast, in several other physiologic or pathologic states that involve a catabolic response in muscle, predominant catabolism (or damage) usually occurs in one or the other fiber types.

In acute infectious disease as in other stressful states, negative nitrogen balance often develops. Skeletal muscle protein degradation accelerates to provide amino acid substrate for hepatic gluconeogenesis, while muscle protein synthesis decreases (6, 26, 27). This combination of metabolic events results in reduced muscle mass (26) and impaired muscle function (1, 10, 11). There are reports in humans that the activities of oxidative and glycolytic enzymes of skeletal muscle, which are correlated with the capacity to perform exercise (15), may become transiently depressed as a result of infection (2, 3).

A search of the literature revealed very few animal experiments concerning effects on oxidative or glycolytic potentials of striated muscle in infection; no studies of muscle lysosomal enzyme activities were found. Kun and Miller (18) reported an inhibition of succinic dehydrogenase but not of cytochrome c oxidase activity in rabbit skeletal muscle by meningococci and Salmonella endotoxin. In a later multi-organ metabolic study of Streptococcus pneumoniae infection in the rabbit, Guckian (13) found no impairment of glycolysis or the oxidation of palmitate, glucose and pyruvate in skeletal and heart muscle.

The purpose of the present investigation was to study effects of bacterial infection on oxidative, glycolytic and lysosomal enzyme activity in skeletal muscle using three different standardized bacterial infections in the rat, i.e., those caused by Francisella tularensis, Salmonella typhimurium or S. pneumoniae. Muscle protein and DNA concentrations were determined. Red and white muscle tissue were studied separately, since they have different metabolic and physiologic properties. Thus, red (slow twitch, type I) muscle fibers contain more mitochondria and are surrounded by a denser net of capillaries than the white (fast twitch, type II) fibers. The latter, instead, have a larger

glycolytic capacity. Red fibers are adapted to long time, low intensity type exercise, whereas the white fibers are recruited predominantly for short time, high intensity efforts (15). If alterations predominate in one of the other fiber types, different patterns of change in physical capabilities may result.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Taconic Farms, Germantown, N.Y.) weighing 200-300 g were maintained on a commercial diet (Wayne LabBlox, Allied Mills, Inc., Chicago, Ill.) until the beginning of an experiment and were housed in rooms maintained at 23 ± 1°C. The initial mean body weights (± SE) of the rats were for the tularemia infection, 256.4 ± 2.5 g, pneumococcal infection, 235.6 ± 3.9 g and Salmonella infection, 273.1 ± 2.6 g.

Infections. Rats were inoculated intraperitoneally (i.p.) with 2.20×10^7 virulent colony-forming units (CFU) of unwashed, F. tularensis, live vaccine strain (LVS) that had been grown on solid, fortified glucose-cysteine-blood agar or with 3.20×10^8 CFU of unwashed, nutrient agar-grown, virulent S. typhimurium, or subcutaneously (s.c.) in the nape of the neck with 2.95×10^4 CFU of nonwashed S. pneumoniae, type Ia5, that had been cultivated on brain-heart infusion broth (19). Control rats were administered similar volumes of sterile tryptose saline. Food was withdrawn from both inoculated and control rats after the inoculation, but water was supplied ad libitum.

Sampling. Six infected and 6 control rats were studied in each infection. Rats were killed 48 h after inoculation except for the tularemia infection, for which the sacrifice time was 72 h. These timings were found to represent the peak of each illness. Rectal temperatures were recorded at that time using a thermocouple.

Rats were rendered unconscious by a blow to the head prior to decapitation, which took 1-2 sec. Blood was then allowed to run into plastic tubes and clot. The quadriceps muscle was then quickly removed and put on ice, after which muscle samples were carefully selected to avoid fibrous tissue. Samples from the proximal deep and distal superficial parts of that muscle represented predominantly red and

white tissue, respectively. After cutting to small pieces with scissors, the samples were immediately weighed and homogenized in 33 times (w/v) ice-cold 0.15 mol/liter KCl, 6 mmol/liter EDTA, 50 mmol/liter $KHCO_3$, pH 7.4, using manual all-glass homogenizers. This procedure was performed at 0 to 4°C.

Assays. The homogenate was used for determination of the activities of cytochrome c oxidase (CYTOX; E.C. 1.9.3.1) (23), citrate synthase (CS; E.C. 4.1.3.7) (22), glyceraldehyde-3-phosphate dehydrogenase (=triose-phosphate dehydrogenase, TPD; E.C. 1.2.1.12) (5) and the following acid hydrolases, using methods except that of cathepsin D essentially as described by Barrett (4): p-nitrophenyl phosphatase (p-NPPase; E.C. 3.1.3.2), acid ribonuclease II (RNase; E.C. 3.1.4.22) β-glucuronidase (GUase; E.C. 3.3.1.31), arylsulphatase A (ASase; E.C. 3.1.6.1), cathepsin C (cat C; E.C. 3.3.14.1) and cathepsin D (cat D; E.C. 3.4.23.5) (8). Total muscle protein (19) and DNA (25) were determined.

All assays except those for RNase, GUase and cat C, protein and DNA were performed immediately. The latter were performed on homogenates that had been frozen and thawed. Since DNA showed no significant change and strictly standardized timings of procedures were employed, all measured variables were calculated and expressed per gram "wet" muscle. Serum was used for determination of zinc according to the method of Pekarek et al. (21).

Statistics. Comparisons were made and correlation coefficients tested by means of Student's t-test, which compared control and experimental groups in each illness.

RESULTS

Rats responded to the infections in a manner expected from previous studies (20). The severity of fever and serum zinc depression at the time of killing are shown in Fig. 1. Enzyme activities and protein and DNA concentrations in muscle of control rats are given in Table 1.

In the tularemia and Salmonella infections the activities of CYTOX and CS were decreased to 65 to 83% of corresponding control values, whereas in the pneumococcal infection alterations were insignificant (Fig. 2). In tularemia, CYTOX and CS decreases were somewhat greater in white than in red muscle, but for the other infections no such difference was evident. Although statistically significant only for tularemia, TPD activity was reduced in all three infections. On a percentage basis, the effect of infection on TPD activity, a reduction to 30 to 75% of activity in controls, was more pronounced than the effects on CYTOX and CS activity.

The acid hydrolases showed increased activity only in tularemia, with a pattern approximately similar in both red and white fibers. The muscle protein concentration was significantly reduced only in tularemia. However, DNA concentrations were unaltered in the three infections (Fig. 2).

Since the most pronounced alterations were found in tularemia, correlation coefficients were calculated to compare the responses among the enzymes of red and white muscle. Some of the more meaningful correlations are shown in Fig. 3. In both red and white muscle, TPD was negatively correlated with several of the acid hydrolases and did not correlate with changes in protein concentration. Significant correlations

were found only in infected rats; no trends of correlation were discerned in the control group (Fig. 3a).

Citrate synthase correlated negatively with only a few of the acid hydrolases (Fig. 3b), but, on the other hand, a positive correlation with protein concentration was found in both red and white muscle. CYTOX showed similar positive correlations with protein as did CS, but CYTOX correlated with none of the acid hydrolases.

Among the acid hydrolases, GUase, pNPPase, and cat D most frequently produced correlation coefficients of 0.5 or more when compared with other acid hydrolases in either red or white muscle. In contrast, ASase changes, the other extreme, did not correlate with any other acid hydrolase. On the whole, the acid hydrolases were activated during tularemia in a similar pattern in red and white muscle and, for each enzyme, a correlation coefficient of at least 0.5 was obtained when activities in red and white muscle were compared. Similar correlations were also recorded for CYTOX, CS, TPD and protein.

DISCUSSION

In the generalized bacterial infections used in the present study, a decrease was often found in the activities of glycolytic and oxidative enzymes in skeletal muscle. The variable extent of the reduction in enzyme activity may be explained by differences in the etiology and/or the duration of each infection. For tularemia, in which the most significant alterations were recorded, the rats had been infected for 72 h at the time of sampling, whereas, for the other infections, the corresponding time was 48 h. In acute infections of man, a negative nitrogen balance begins only after the onset of fever, but the cumulative loss of body nitrogen becomes increasingly evident as time

elapses (1, 6). The more pronounced effects recorded in the Salmonella infection than in the pneumococcal infection, although both lasted for 48 h, may be ascribed to differences in pathogenic responsiveness to the bacteria or their components. An endotoxin effect on muscle enzymes may have been present in the Salmonella infection, since the host's response to the infection as measured by fever and serum zinc depression were similar in both the Salmonella and pneumococcal infections (Fig. 1).

In skeletal muscle, as in other tissues, acid hydrolases become activated in states associated with increased tissue degradation or turnover, such as fasting, disuse (7) or physical training (24). With several exceptions, activities of acid hydrolases were increased in the present study only in tularemia. This was the only infection to show any significant decrease of muscle protein concentration. However, correlations between the acid hydrolases, including the proteases cat C and D on the one hand, and protein concentration on the other, were poor. This may be explained by the fact that these acid hydrolases may be involved primarily in the degradation of sarcoplasmic protein (16, 24), whereas myofibrillar protein predominates in the muscle cell (14). In support of this concept, significant correlations were found between the activity of acid hydrolases and the sarcoplasmic enzyme TPD during tularemia in rats (Fig. 3a).

Cytochrome c oxidase is an integral protein of inner mitochondrial membrane. This anatomical localization may account for a lack of significant correlations with the acid hydrolases. On the other hand, CYTOX was significantly correlated with protein concentration, raising the possibility that similar molecular mechanisms might account for both CYTOX inactivation and muscle cell protein degradation (Fig. 3b).

The activities of several acid hydrolases of skeletal muscle are increased concomitantly in response to various influences, e.g., fasting, immobilization and physical training (7, 24). We found similar increases during tularemia in the present study, although comparisons between some enzymes correlated more closely than those among others.

In the present study a strict "every other" order of test and control rats was followed and an exact timing was employed in sampling. Analytic results were calculated per gram of "wet" muscle. The unaltered concentration of muscle DNA in all three infections confirms the value of DNA as an "internal" reference standard in muscle. The somewhat larger variance in the results of TPD than in those of the other enzymes may, at least in part, be explained by the possibility that TPD activity, being rather labile, might have decreased somewhat prior to the period of analysis. However, with the procedures employed, any decreases would have been comparable in both the experimental groups and their controls.

In the present study, alterations in enzyme activity in response to infection generally occurred to a similar extent in both red and white muscle tissues. This agrees with findings in a previous human study of skeletal muscle lactate dehydrogenase isozymes in infection that gave indirect evidence that both fiber types are similarly affected (12). This is the first direct evidence that skeletal muscle degradation occurs at roughly similar rates in red and white muscle during bacterial infection. These results suggest that the metabolic responses to infection are sufficiently generalized that red and white fibers show comparable changes. This similarity contrasts with differences in the responses of these types of fiber in certain other diseases or stresses involving muscle. Thus, in a study by Vihko et al. (24), physical

training caused activation of a larger number of acid hydrolases in red muscle tissue than in white, although the training-induced build-up of citrate synthase activity was quite as marked in the white fibers, indicating considerable recruitment and loading of both fiber types. Similarly, denervation, ischemia or vitamin E deficiency may predominantly affect red fibers (24), whereas rheumatoid arthritis (9) and alcoholism (17) cause a selective hypotrophy of white fibers. Therefore, the present finding of similar responses in white and red muscle in terms of activation of acid hydrolases and protein degradation suggests that in infection, several or different metabolic pathways are employed to evoke these responses compared to those at work in the aforementioned states. These infection-induced responses appear to be unassociated with the specific morphologic, physiologic and biochemical properties that constitute the different muscle fiber types. This concept agrees with the hypothesis that the response of muscle to infection is primarily catabolic, whereas, that of training exercise contains both a small catabolic component and a predominating anabolic response (24), which may be independently mediated in muscle.

In conclusion, bacterial infections of different etiologies caused a decrease in the activities of oxidative as well as of glycolytic enzymes of skeletal muscle, and a simultanous activation of several lysosomal enzymes. All effects occurred to similar extents in red and white muscle tissue but were most pronounced in tularemia, the infection that lasted longest. The glycolytic enzyme in the sarcoplasm, showed an earlier and more pronounced response than did oxidative enzymes with an intramitochondrial location. Negative correlations existed between activities of glycolytic and lysosomal enzymes, whereas positive correlations were produced by oxidative enzyme activities and total

intracellular protein. The equally pronounced catabolic responses to bacterial infections in red as in white muscle fibers suggest that the mechanisms which mediate these responses in infection differ from those in other states, such as training or denervation, which include a catabolic component in muscle.

ACKNOWLEDGMENTS

Financial support was obtained from the Swedish Armed Forces Medical Research Board (FOA) (Grant No. FMFD 78/79). The authors are indetted to Dr. Lars Pilström for valuable advice and to Mrs. Ninni Rosén for excellent technical assistance.

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TABLE 1. Normal enzyme values in rat skeletal muscle. Means + SE of sham-inoculated control rats from all three infections are given (n = 18)

Component	Unit per g wet muscle	Red muscle	White muscle
Enzymes			
Oxidative			
Cytochrome c oxidase (CYTOX)	$\mu g\ O_2 \times min^{-1}$	81.6 ± 2.3	9.9 ± 0.5
Citrate synthase (CS)	$\mu moles \times min^{-1}$	4.02 ± 0.06	1.00 ± 0.04
Glycolytic			
Triose-phosphate dehydrogenase (TPD)	$mmoles \times min^{-1}$	5.0 ± 0.7	91.9 ± 14.8
Lysosomal acid hydrolases			
p-Nitrophenyl phosphatase (p-NPPase)	$\mu moles \times 10^{-2} \times min^{-1}$	93.8 ± 2.5	68.4 ± 2.3
Acid ribonuclease II (RNase)	$\mu moles \times 10^{-3} \times min^{-1}$	65.6 ± 4.4	67.8 ± 4.9
[illegible] (GPase)	$\mu moles \times 10^{-3} \times min^{-1}$	26.9 ± 0.7	22.4 ± 0.7
[illegible]sphatase A (ASase)	$\mu moles \times 10^{-3} \times min^{-1}$	21.3 ± 2.1	12.4 ± 0.8
[illegible] (CPase)	$\mu moles \times 10^{-3} \times min^{-1}$	76.3 ± 3.3	45.2 ± 2.6
[illegible] (CPase)	μg albumin $\times 10^{-1} \times min^{-1}$	21.8 ± 0.9	10.4 ± 0.9
[illegible]	mg	94.0 ± 2.7	130.0 ± 6.3
[illegible]	%	0.76 ± 0.06	0.79 ± 0.07

FIGURE LEGENDS

FIG. 1. Rectal temperature and serum zinc concentration in S. pneumoniae-, S. typhimurium-, and F. tularensis-infected rats (●) and controls (○). Group mean ± SE values are shown.

FIG. 2. Specific activities of enzymes and protein and DNA concentrations in per cent of controls in red and white muscle in (a) S. pneumoniae, (b) S. typhimurium, and (c) F. tularensis infections. 1 = cytochrome c oxidase (CYTOX), 2 = citrate synthase (CS), 3 = triose-phosphate dehydrogenase (TPD), 4 = p-nitrophenyl phosphatase (p-NPPase), 5 = acid ribonuclease II (RNase), 6 = β-glucuronidase (GUase), 7 = arylsulphatase A (ASase), 8 = cathepsin C (cat C), 9 = cathepsin D (cat D), 10 = protein, and 11 = DNA. Asterisks indicate statistically significant differences, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

FIG. 3. (a) Triose phosphate dehydrogenase (TPD) activity in relation to activities of the lysosomal enzymes GUase, pNPPase, RNAse and cat D in white muscle of F. tularensis-infected rats (●) and controls (○). r = correlation coefficient. Asterisks indicate the probabilities as shown in Fig. 2. (b) Citrate synthase (CS) activity in relation to activities of GUase and pNPPase and protein concentration, and cytochrome c oxidase (CYTOX) activity in relation to protein concentration in red muscle of F. tularensis-infected rats (●) and controls (○).

Figure 1

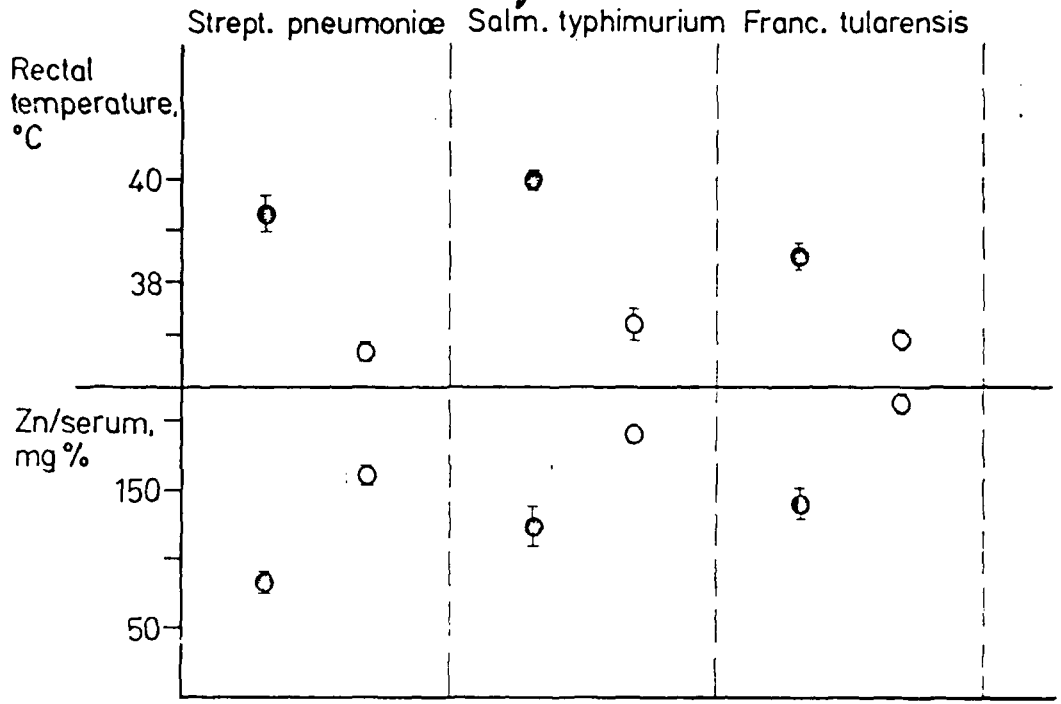

Strept. pneumoniæ
Salm. typhimurium
Franc. tularensis
Rectal temperature, °C
40
38
Zn/serum, mg %
150
50

Figure 2b

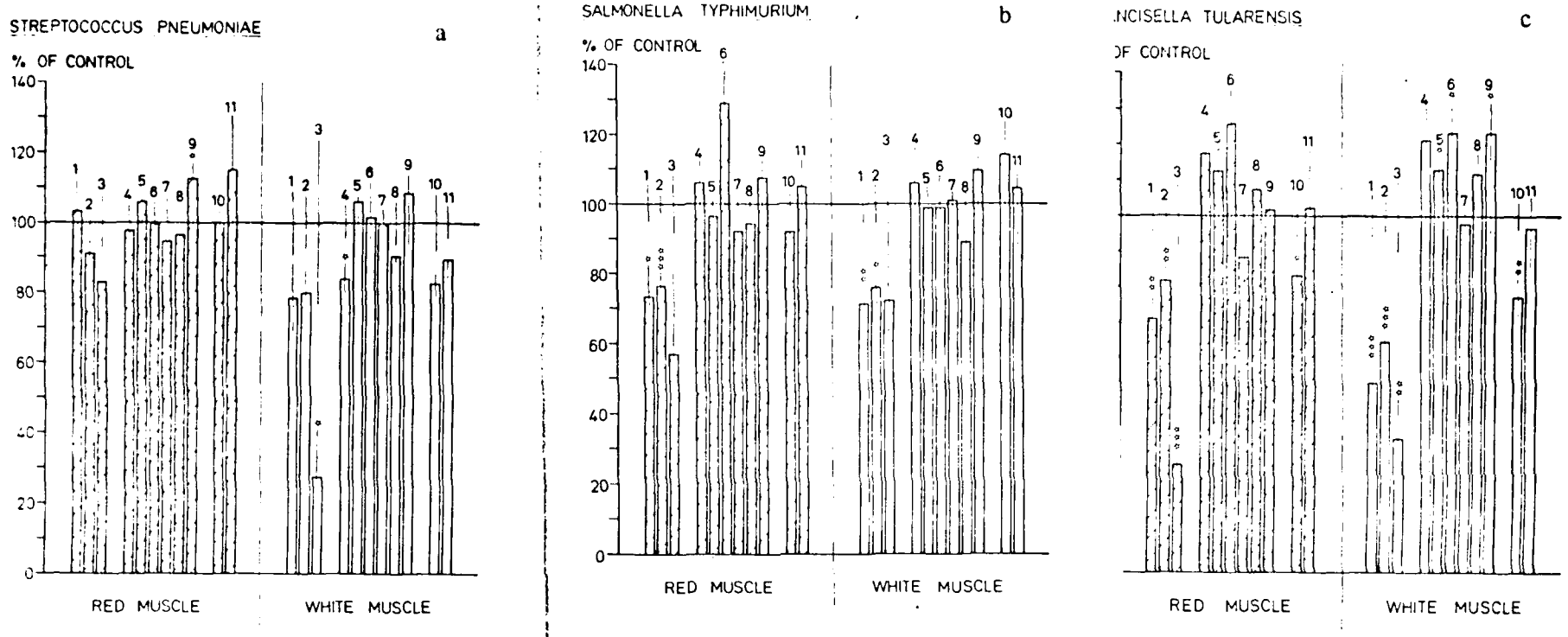
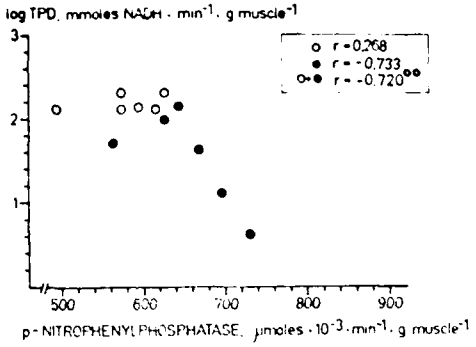
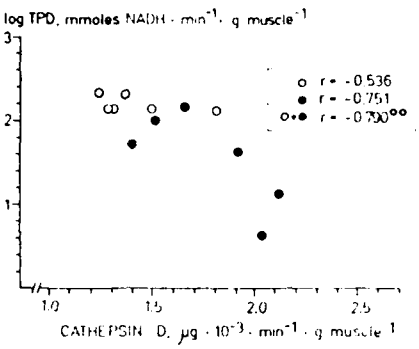
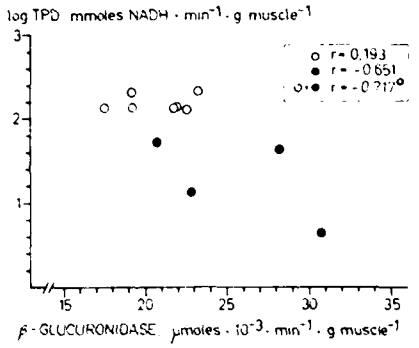

STREPTOCOCCUS PNEUMONIAE
a
% OF CONTROL
140
120
100
80
60
40
20
0
RED MUSCLE
WHITE MUSCLE
SALMONELLA TYPHIMURIUM
b
% OF CONTROL
RED MUSCLE
WHITE MUSCLE
.NCISELLA TULARENSIS
c
OF CONTROL
RED MUSCLE
WHITE MUSCLE

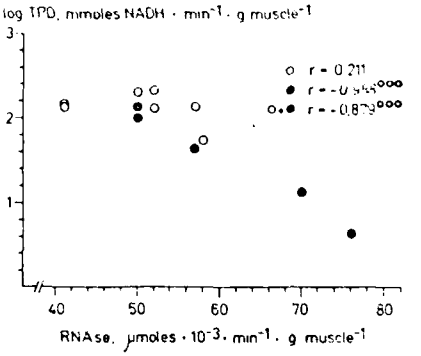
Figure 3

A.

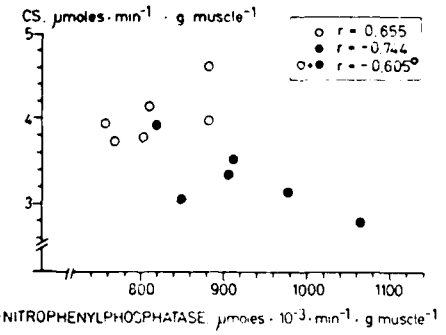

log TPD, mmoles NADH · min⁻¹ · g muscle⁻¹
r = 0.268
r = -0.733
r = -0.720°°
p-NITROPHENYLPHOSPHATASE, μmoles · 10⁻³ · min⁻¹ · g muscle⁻¹

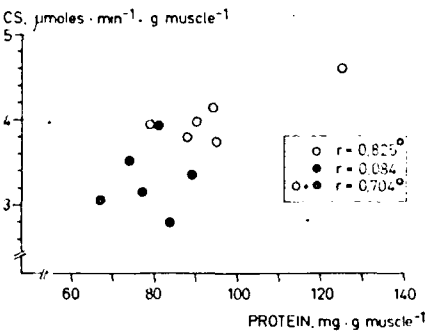

log TPD, mmoles NADH · min⁻¹ · g muscle⁻¹
r = -0.536
r = -0.751
r = -0.790°°
CATHEPSIN D, μg · 10⁻³ · min⁻¹ · g muscle⁻¹

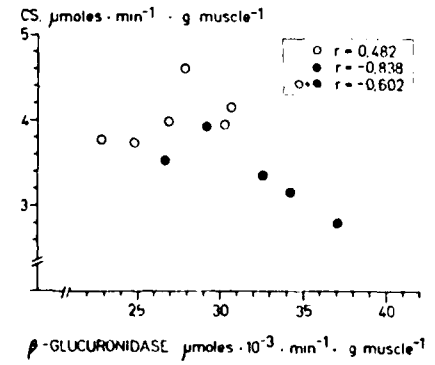

log TPD, mmoles NADH · min⁻¹ · g muscle⁻¹
r = 0.193
r = -0.651
r = -0.717°
β-GLUCURONIDASE, μmoles · 10⁻³ · min⁻¹ · g muscle⁻¹

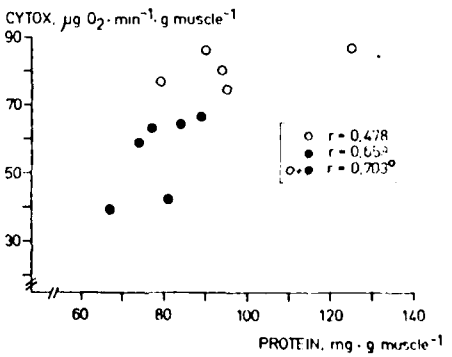

log TPD, mmoles NADH · min⁻¹ · g muscle⁻¹
r = 0.211
r = -0.855°°°
r = -0.879°°°
RNAse, μmoles · 10⁻³ · min⁻¹ · g muscle⁻¹

B.


CS, μmoles · min⁻¹ · g muscle⁻¹
r = 0.655
r = -0.744
r = -0.605°
p-NITROPHENYLPHOSPHATASE, μmoles · 10⁻³ · min⁻¹ · g muscle⁻¹


CS, μmoles · min⁻¹ · g muscle⁻¹
r = 0.825°
r = 0.084
r = 0.704°
PROTEIN, mg · g muscle⁻¹


CS, μmoles · min⁻¹ · g muscle⁻¹
r = 0.482
r = -0.838
r = -0.602
β-GLUCURONIDASE, μmoles · 10⁻³ · min⁻¹ · g muscle⁻¹


CYTOX, μg O₂ · min⁻¹ · g muscle⁻¹
r = 0.478
r = 0.653
r = 0.703°
PROTEIN, mg · g muscle⁻¹

END

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